AGITATION-AERATION IN THE LABORATORY AND IN INDUSTRY

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It is impossible to aerate all portions of a culture fluid without some degree of stirring, and therefore agitation-aeration comprises a single topic. As suggested by the title, this review will concern the physical rather than the biochemical aspects of oxygen uptake. Its aim is to draw together and to interpret for microbiologists information which is now widely scattered through the biological and engineering literature. Emphasis will be given to principles and techniques rather than to particular applications of oxygen transfer. A point to be stressed is that laboratory workers as well as fermentation engineers should seek accurate definition of the aeration in a particular piece of equipment so that experimental results may be readily reproduced in other laboratories. At present, many of the same scientists who would shrink from describing a culture medium as "strongly acid" or "moderately warm", are nevertheless content to characterize its aeration by such qualitative phrases as "intense" or "mild".

Much of the current interest in aeration can be traced to the changing character of the fermentation industry itself. During the past decade, products of oxybiontic synthesis, antibiotics and the like, have largely displaced in importance such classical anaerobic products as ethanol and butanol. The trend is likely to continue, making ever more valuable our knowledge of cell respiration and aerobic tissue culture.

The new era in fermentation was heralded by the introduction in 1933 of a shake-flask technique by Kluyver and Perquin (1). Developed initially for culturing molds in the laboratory, the "Schuttelkultur" method was soon adapted to a factory scale by using horizontal rotating drums (2) or vertical deep-tank fermenters with spargers and mechanical stirrers to disperse the air. The latter type is now standard apparatus for the submerged production of vitamins and antibiotics from a variety of bacteria, molds, and actinomycetes. Its historical development has been reviewed by Hromatka and Ebner (3).

In laboratory work the provisions for aeration have usually been inadequate as shown by studies of Rahn and Richardson (4). The neglect is rather surprising, because the role of dissolved

oxygen in altering cellular metabolism and in promoting growth has been known since the time of Pasteur. Nevertheless, even recent books on physiology (5, 6, 7) give scant attention to the matter of oxygen supply, owing perhaps to uncertainty regarding its classification as a physical or a chemical factor affecting growth. Allen (8) has summarized the situation by commenting that, "For the growth of bacteria there are few data which are complete enough to deserve theoretical treatment, and which provide assurance that the investigator was measuring anything more significant than the rate of diffusion of oxygen into solution."

I. OXYGEN SUPPLY AND DEMAND

1. *The solubility of oxygen.* Oxygen is a rather insoluble gas, water at 20 C and in an atmosphere of air holding only about 9 parts per million of oxygen. As the temperature is raised, oxygen, like any other gas, becomes *less* soluble. At 37 C for example, water in perfect contact with air contains less than 7 ppm of oxygen. The solubility of oxygen is substantially independent of the total pressure and the presence of other gases. It is, however, directly proportional to the partial pressure of oxygen in the gas phase. This fact, known as Henry's law, may be written as

$$C^* = \frac{1}{H'} p_{O_2} \qquad (1)$$

where H′ is a Henry's law constant based on molar concentration,[1] and the partial pressure is expressed in atmospheres. The asterisk on C denotes concentration in the liquid at equilibrium. Henry's law predicts that in an atmosphere of pure oxygen, water will dissolve about five times as much oxygen as in air, where the partial pressure is only 0.21 atmospheres.

Umbreit, Burris, and Stauffer (9) present data on the effect of dissolved salts on oxygen solubility. These data and others in the literature are often expressed in terms of a Bunsen coefficient, α. The Bunsen coefficient should be multiplied by 1,000/22.4 to get H′, which turns out to be more convenient in aeration studies. For nutrient broth saturated with air at 25 C, C* is about 0.20 mM per liter, so that H′ is close to unity.

Since the respiratory enzymes are imbedded within aqueous protoplasm, microorganisms can utilize only dissolved oxygen, even if grown at an air-water interface. Furthermore, oxygen is so insoluble that there exists at any time only a small reservoir of it in solution. Upon this reservoir the microorganisms are continuously drawing, and into the reservoir there must flow a fresh supply of oxygen to balance the demand. Because the reservoir has such low capacity, and because the oxygen demand of microbial tissue is so high, the rate of supply must at least equal the rate of demand in every portion of the culture fluid. Otherwise, there will be local or temporary depletion which damages the respiring cells. Such damage was dramatically shown by Hromatka, Ebner and Csoklich (10), who found that interruption of the air flow to *Acetobacter* for 15 seconds caused death and disrupted metabolism.

Another consequence of low solubility is the limit it imposes on the rate of oxygen supply to the culture fluid. To see this restriction more clearly let us revert again to the analogy of a reservoir. Imagine that the supply comes from an ocean of oxygen held at the same level as the top of the reservoir, and that transfer is accomplished by some sort of siphon arrangement. The rate of inflow will then depend on the relative depletion of the reservoir. Because the latter is so shallow (low solubility), no large driving force can ever develop, and even the maximum supply rate is rather low.

These considerations may seem very elementary; nevertheless they point up some common misstatements of fact. One of these is that the culture fluid must be kept saturated with air at all times. Strictly speaking such a condition is impossible with respiring cultures. Nor can oxygen be supplied at a rate faster than it is being consumed, for as the reservoir becomes full the rate of supply falls off to zero.

2. *Oxygen demand.* Happily for microbiologists, there is no need to keep cultures saturated with air. Cell respiration proceeds at a rate which is independent of the dissolved oxygen concentration so long as the latter remains above a critical value. For unicellular organisms at least, C_{crit} is extremely low. Typical values are given in table 1.

Below the critical oxygen level, respiration rate

[1] Strictly stated the solubility should be expressed as a mole fraction, but for slightly soluble gases there is little error in taking molarity as proportional to the mole fraction. The units for H′ as used here are liter-atmospheres/millimole O_2.

TABLE 1

Typical values of C_{crit} in the presence of substrate

Organism or Tissue	Temperature C	C_{crit} mM/liter	Equivalent O_2 Tension, mm Hg	Reference
Luminous bacteria	24	*ca* 0.010	*ca* 7.5	11
Azotobacter vinelandii	30	0.018–0.049	15–40	12
Escherichia coli	37.8	0.0082	7.5	13
	15.5	0.0031	1.9	13
Yeast	34.8	0.0046	4.0	14
	20.0	0.0037	2.5	14
Penicillium chrysogenum	24	*ca* 0.022	*ca* 16	15
Kidney slices	37	*ca* 0.85	*ca* 760	16

falls off in hyperbolic manner. In a series of beautifully designed experiments with yeast cells, Winzler (14) demonstrated that at low oxygen tension respiration rate is limited by unsaturation of the enzyme surfaces rather than by slow diffusion of oxygen into the protoplasm. He made use of the fact that carbon monoxide acts as a competitive inhibitor, displacing oxygen from the cytochrome oxidase of yeast. With CO–O_2 mixtures, a falling respiration rate was observed at oxygen tensions so high that diffusion of O_2 could not possibly have been rate limiting. Nevertheless such CO–O_2 rate curves could be superimposed upon the rate curve taken at low O_2 concentration with no CO if correction was made for competitive effects. Thus the underlying mechanism for the decrease in respiration rate at low oxygen concentration was proved to be enzyme unsaturation. A similar mechanism probably occurs in all unicellular microorganisms although a recent report of Longmuir (16a) reopens the whole question at least as regards the bacteria. Longmuir, apparently unaware of the pioneer work of Winzler, did not use the CO technique. Rather he observed essentially that C_{crit} varied in regular fashion with the size of the organism, larger bacteria exhibiting a higher critical oxygen level. Although these facts provide circumstantial evidence for diffusion-controlled uptake at low oxygen tension, the possibility remains that all trends arose from different oxygen affinities in the cytochrome systems tested. As pointed out by Smith (16b) the variations in bacterial cytochromes are complex. Diffusion of oxygen is more likely to become rate limiting in multicellular organisms or clumps of cell tissue. Such possibilities are discussed by Berry (17) and by Stevenson and Smith (16) among others.

Above the critical oxygen concentration where neither diffusion nor unsaturation controls, it is presumed that the rate at which reduced substrate can be supplied sets the pace of respiration. The respiratory "rebound" which occurs when air is readmitted to cells held anaerobic for a time provides evidence for this explanation (18). Alternatively, the quantity of oxygen transferring enzyme may be rate limiting. These and other aspects of oxygen uptake by cells and tissues are fully treated in reviews by Goddard (19), Tang (20, 21), and Kempner (22).

Surprisingly little is known about the peak oxygen demand of microbial cultures which are actively growing in a rich medium. While measurements of Q_{O_2} abound,[2] these usually reflect the behavior of cells under artificial conditions where there is no growth and where simple substrates are present in low concentration. Therefore, they cannot serve as a reliable index of aeration requirements (23). Perhaps the most complete study of oxygen uptake is that by Clifton (24) who worked with enterobacteria. He showed that the uptake per cell is highest in very young cultures, and falls off as the cells age. A young culture, however has such a low cell concentration that total demand is slight. The peak demand of a culture is reached during the phase of declining growth rate when the product of cell concentration and Q_{O_2} is highest. In general one may write

$$\text{Rate of demand} = C_c Q_{O_2} \qquad (2)$$

where C_c is the cell concentration, and Q_{O_2} is the specific respiration rate on a molar basis ($Q'_{O_2} = Q_{O_2}/22.4$). Some typical demand rates are collected in table 2. These should not be considered the maximum possible rates since all values are strongly dependent on cultural conditions as well as on the particular strain of organism used.

Among the environmental factors that can affect the total demand rates are:

[2] Q_{O_2} is the commonly used quotient for oxygen uptake, defined as microliters O_2 taken up per mg dry weight of tissue per hour.

1. Concentration of sugar or other nutrient (affects C_c).

2. Accumulation of toxic endproducts or loss of volatile intermediates (affects C_c).

3. Amount and nature of nitrogen sources, mineral salts, and accessory growth factors (affect primarily C_c but also Q'_{O_2}).

4. The supply of oxygen itself (affects primarily C_c but also Q'_{O_2}).

The interplay of these factors is best illustrated in the work of those who have tried to prevent any one condition from limiting the maximum population (33, 49, 94a). For yeast, Maxon and Johnson (49, table 2) demonstrated that with a poor medium (1% glucose) and inadequate aeration the oxygen demand ranged from 15.0 to 18.5 mM/(liter)(hr), whereas with a rich medium (10% glucose) and high aeration rates the demand rose to 296–342. The change to a superior environment brought about a 10-fold increase in cell concentration along with a doubling of specific respiratory activity. Hixson and Gaden (26) reported a peak oxygen demand of only 10–15 for cultures of *Saccharomyces cerevisiae*. Aeration was probably adequate in their experiments, but sugar concentrations were low and there may have been nutritional deficiencies because the Q'_{O_2} was only 2.5 as compared to an average of 7.7 for the cells grown by Maxon and Johnson. Differences in the strains of yeast may also account for the lower oxygen demands observed by Hixson and Gaden.

3. *Measuring oxygen demand.* The net rate of oxygen uptake by a cell suspension may be limited either by diffusion into the liquid or by the demand of the organisms. When measuring Q_{O_2} the rate of supply must be eliminated as a controlling factor, and for this reason Warburg type respirometers are shaken rapidly so as to provide a large gas-liquid interface. Umbreit, Burris, and Stauffer (9) have reviewed the effect of shaking. They note for example that one hundred 2 cm strokes per minute are necessary if the respiration rate is as high as 300 microliters of oxygen per hour.

Another method for measuring oxygen demand is variously described as polarographic, voltammetric or amperometric. It makes use of a dropping-mercury electrode to measure directly the concentration of dissolved oxygen in a cell suspension. No assumption of "equilibration" with the gas is necessary. In fact, uptake data are taken by cutting off the air supply to a cell suspension and noting the course of depletion in the oxygen reservoir. The rate of consumption can usually be determined in 5 minutes or less, and therefore the method is especially suited for use with actively growing cultures.

Polarographic determinations depend upon the fact that at an applied voltage of about 0.6 the flow of a diffusion current to freshly formed mercury surfaces is proportional to the concentration of dissolved oxygen. The mercury electrode is immersed in the test suspension, which in turn is connected through a salt bridge

TABLE 2

Peak oxygen demands of active cultures—Estimations from published data[a]

Type of Culture	Total[b] Demand, mM O_2/(liter)(hr)	Reference
Activated sludge (domestic sewage)	1–2	25
Escherichia coli, Aerobacter aerogenes	5–8	24
Yeast	10–15	14, 26
	340	49
Streptomyces griseus (streptomycin)	15	27, 28
Ustilago zeae (ustilagic acid)	16	29
Penicillium chrysogenum (penicillin)	20–30	15, 30, 31
Aspergillus niger (citric acid)	28	29
Aspergillus niger (α-amylase)	56	29
Acetobacter sp.	90	32
Azotobacter vinelandii	260	33

[a] All the values of "oxygen uptake" reported were critically examined to make sure that they represented the rate of oxygen demand and not merely the rate of oxygen supply to a particular piece of test equipment.

[b] A prolongation of exponential growth usually, but not always, brings about an increase in the peak demand on a "per liter" basis, and some of the low values reported here undoubtedly reflect experimental conditions that led to a low yield of cells. For azotobacter and yeast (49), however, rich nutrient solutions were used in an effort to get maximum cell crops, and the oxygen demands were correspondingly high. In fact, the data on yeast were obtained during a continuous fermentation which provided somewhat higher cell populations than would ordinarily be obtained in batch culture (49).

to a calomel half-cell. Galvanometer readings of a slide-wire circuit can be calibrated as a linear function of the oxygen concentration. Polarography was adapted for use in biology by Baumberger (34), and its principles have been described briefly by Petering and Daniels (35) and by Umbreit, Burris, and Stauffer (9). Skerman and Millis (36) present a more complete and critical discussion, which should be consulted by anyone intending to use the method.

Instead of falling mercury droplets, a platinum microelectrode may be used for voltammetric measurements provided the pH is above 5.0; in solutions which are more acid, hydrogen ions discharge onto the platinum. Polarization effects must be avoided, and therefore solid electrodes are usually rotated. A clever alternative has been devised by Olson, Brackett, and Crickard (37), however, who impressed a square-wave potential on the platinum electrode and then arranged to take galvanometer readings only during each half-cycle, thereby simulating the effect of a mercury droplet. If such an instrument could be made more rugged and stable, it would find application in industry for continuously monitoring dissolved oxygen content.

4. *Transport of oxygen to the enzyme.* In its pathway from the gas phase to an enzyme surface, oxygen encounters various resistances in series, the principal ones consisting of more or less stagnant films. Through these the transport is mainly by diffusion. Consider first the supply-side resistances *i.e.*, those which slow down the rate at which oxygen can enter solution or at which it can "flow into the reservoir". There are two films, one between the bulk of the gas and the gas-liquid interface, and the other extending from the interface to the bulk of the liquid. Such films may be anywhere from microns to millimeters thick, depending upon the degree of turbulence and the physical properties of the fluids. At the interface itself one can also postulate a barrier since only those molecules which possess sufficiently high energy can penetrate into the liquid phase. The remainder suffer reflection back into the gas phase (38).

Concentrations in the bulk of the gas phase and in the bulk of the liquid phase are assumed to be uniform. This is simply another way of stating that there is negligible resistance in the bulk phases. Of course in stagnant pools of liquid or gas or in agar medium, one cannot make such an assumption. However, even mild mechanical agitation provides rapid mixing of dissolved solutes (39), and the stirring provided by rising air bubbles is usually sufficient to dispel all gradients, even those within the bubbles (26, 40).

On the demand side of the oxygen reservoir, the resistances are: (a) the liquid film around individual cells or cell clumps; and (b) intracellular and intraclump resistances. Insofar as these are significant, the measured values of Q_{O_2} will misrepresent the enzymatic behavior of cells. Discussion will be deferred until later in this review because our major concern is with supply-side resistances, over which the investigator has more control.

The foregoing picture of oxygen transfer represents a special case of gas absorption, which is one of the so-called unit operations studied intensively by chemical engineers (41). Some of the first applications to biology were made by Roughton (42, 43) whose special concern was the gaseous exchange of red blood cells in aqueous suspension. More recently Bartholomew *et al.* (27), Hixson and Gaden (26), and Wise (44) have applied the concepts to microbial respiration.

II. SUPPLY-SIDE RESISTANCES

1. *The absorption equation.* It has already been stated that the rate of dissolution of oxygen is proportional to the depletion of dissolved oxygen in the liquid. The rate is also proportional to the interfacial area, a, and therefore one may write for a unit volume of culture fluid,

$$\text{Rate of absorption} = K_L a\ (C^* - C_L) \quad (3)$$

where C_L is the actual concentration of oxygen in the liquid. The proportionality constant, K_L, may be looked upon as an over-all conductance. Its reciprocal, $1/K_L$, an over-all resistance, is equal to the sum of the separate resistances residing in the gas film, the interface, and the liquid film. When these resistances are large, K_L is small and vice versa.

Neither K_L nor a can be evaluated directly as a rule, but the product $K_L a$ can be found indirectly by measuring C_L (voltammetrically), C^* (by Henry's law), and the rate of absorption. Since, in a steady state, the rate of absorption must exactly equal the rate of demand, the left hand member of the above equation is given by

$C_cQ'_{O_2}$ from Equation 2. An explicit equation for K_La in terms of known or measurable quantities is

$$K_La = \frac{C_cQ'_{O_2}}{(C^* - C_L)} \quad (4)$$

where C_c = cell concentration, mg/ml or g/liter
Q'_{O_2} = specific rate of oxygen uptake, μM/(hr) (mg dry wt) or mM/(hr) (gm dry wt)
C^* = equilibrium concentration of dissolved oxygen, μM/ml or mM/liter
C_L = actual concentration of dissolved oxygen, μM/ml or mM/liter
K_La = absorption rate, mM/(hr)(liter)(unit concentration difference), or 1/hr.

Over short time intervals C_c, Q'_{O_2}, and C_L are substantially constant. Even when these quantities vary during fermentation, however, K_La remains nearly fixed. It is unlikely that gases leaving the fermentation unit are appreciably depleted in oxygen, but if so an average C^* should be used (27).

The product K_La provides an over-all measure of the gas absorbing capacity of any fermenter. It is in fact the only satisfactory way to characterize the performance of laboratory or industrial devices. Olson and Johnson (45) were perhaps the first to recognize this when they used K_La values to describe the degree of aeration in shake flasks and in a small stirred fermenter. High values of K_La denote an efficient oxygenating device whereas low values, an inefficient one.

2. *Units of K_La.* Before considering the factors which affect K_La it is worthwhile to review the units of measure, because various investigators have not been consistent in this regard.

Cooper, Fernstrom, and Miller (46) in a famous study of oxygen absorption in stirred tanks reported their results in terms of a K_V which was based on English units and a partial pressure difference rather than the equivalent concentration difference. Still other systems were used by Hixson and Gaden, Bartholomew *et al.*, and Wise. A comparison of these is made in table 3. Not included in table 3 are Streeter's "reaeration coefficient", K_2 (47), and Adeney's "escape coefficient", f (48) which appear occasionally in the literature on sewage treatment. The choice of nomenclature for this review results from the author's conviction that whenever possible coinage of new symbols should be avoided; the term K_La is accepted in the engineering literature (41).

TABLE 3

Comparison of units for absorption capacity

Symbol	Units	Investigator	Factor for Conversion to K_La[a]
K_V	lb moles/(cu ft) (hr) (atm)	Cooper, Fernstrom, Miller (46)	1.68×10^4
k_d	1/hr	Hixson and Gaden (26)	1
k_d	g moles/(ml) (hr) (atm)	Bartholomew *et al.* (27)	1.05×10^6
φ_L	1/min	Wise (44)	60
K_La	1/hr	This review	1

[a] At C^* = 0.2 mM/liter = 6.4 ppm).

Another source of confusion in the agitation-aeration literature arises in the following way. Instead of using the absorption rate constant directly, a number of workers (29, 31, 45, 49) have chosen to multiply it by C^*. This is quite all right; the units then become mM/(liter) (hr) or some equivalent set which signifies the maximum rate of absorption. But a recent article by Karow, Bartholomew, and Sfat (50) proposes that the k_d factor of Bartholomew *et al* (see table 3) be multiplied by P, the *total* pressure. This is inconsistent with the prior practice and tends to confuse the casual reader, who is left with a feeling that the subject matter is inherently difficult.

3. *Methods for measuring K_La.* There are three methods generally used to measure K_La. Two of these involve the polarograph and are described by Wise (44) as the "sampling method" and the "gassing-out method".

In the sampling method, a value of C_L is obtained by quickly placing a sample from the actual fermentation in a polarographic cell and then noting the concentration of dissolved oxygen at various times. A graph of the results is extrapolated to zero time, *i.e.*, the time of sampling. Knowing C_L and Q'_{O_2} (from the slope of the above-mentioned plot), Equation 3 is used to find K_La. In a modified procedure, Hixson and Gaden (26) sampled with a nitrogen-flushed hypodermic syringe which also contained a small amount of phenol to "kill" the respi-

ration. Initial readings on the polarograph then gave C_L directly.

Wise (44) considers that the gassing-out technique is more accurate than the one just described, though not so convenient, because the fermentation vessel must be held out of production during a test. Either broth or a dilute salt solution is placed in the fermentation vessel, and after a preliminary gassing-out with nitrogen, aeration is begun at constant flow rate. At convenient intervals thereafter, the value of C_L is measured voltammetrically by a sampling technique. The time rate of increase in the concentration of dissolved oxygen may be written $dC_L/d\theta$, and therefore equation 3 becomes

$$\frac{dC_L}{d\theta} = K_La(C^* - C_L) \quad (5)$$

This means that the unsteady-state absorption of oxygen follows a logarithmic course, for upon integration equation 5 yields

$$\ln (C^* - C_L) = -K_La\theta + \text{constant of integration} \quad (6)$$

To evaluate K_La it is convenient to plot log $(C^* - C_L)$ against the time, θ, whence

$$K_La = -2.303 \text{ (slope of straight line)} \quad (7)$$

In using this gassing-out method Bartholomew *et al.* (27) located the dropping-mercury electrode within the fermentation vessel, thereby increasing the speed of manipulation.

A third method of measuring K_La is indirect. It involves the carrying out of a sulfite oxidation in the fermentation apparatus. In the presence of copper or cobalt salts, which act as catalysts, the reaction with oxygen or air proceeds rapidly and irreversibly to completion in the liquid phase. The reaction rate is not only much more rapid than the absorption rate, but it is independent of sulfite ion concentration at molarities above 0.015. These features attracted Cooper, Fernstrom, and Miller (46) to the use of sulfite oxidation as a test system for evaluating gas-liquid contactors. To find K_La it is necessary only to titrate the sulfite solution with iodine at intervals during aeration. The rate of absorption found in this manner is K_LaC^* since C_L, the concentration of dissolved oxygen, is zero at all times.

Unfortunately, the absorption of air by sulfite solutions is not so simple as it seems. In fact, considerable confusion has arisen over the interpretation of results from such tests, and many of the issues are still unsettled. Miyamoto (38), one of the early investigators, became convinced that the absorption rate into sulfite was limited primarily by resistance at the interface, a view that has recently been accorded fresh attention (51). On the other hand, some workers (44, 46) have referred to the liquid film as the site of rate limiting diffusion. Wise (52), for example, has accounted for differences between the polarographic and sulfite methods of measuring K_La by assuming finite reaction rates in a stagnant liquid film. Just recently Maxon and Johnson (49) made the unusual claim that absorption in sulfite was gas-film controlled. They offered no supporting arguments other than that the chemical reaction was extremely rapid and of zero order. The complexity of the chemistry involved becomes evident from the fact that in a review by Abel (53) over 100 references are cited.

To use the sulfite method in the presence of broth or cells, Bartholomew *et al.* (27) found it necessary to measure the back-pressure of oxygen polarographically because anticatalysts were present. Apparently Shu (29) did not make this correction, but his results were consistent nonetheless. The sulfite method of finding K_La or K_LaC^* is certainly a convenient one since it involves no special instruments. Results must be interpreted with caution, however, if the vessel under test is to be used for fermentation instead of for sulfite oxidation (52).

4. *Individual resistances and K_La.* The rate-controlling step in oxygen absorption cannot be the gas film. This was proved by Bartholomew *et al.* (27) who measured temperature effects of absorption by sterile fermentation broth. Not only did K_La increase as the temperature was raised from 27 C to 32 C, but the thermal increment or activation energy for the over-all process was 4,200 calories per mole, a value characteristic of diffusional processes through water. If diffusion through a gas film had been controlling, K_La would vary as the square root of the absolute temperature, and this was not observed.

For a slightly soluble gas like oxygen the concentration driving force across the gas film is so much greater than the driving force across the

liquid film that the latter is almost certain to offer more resistance. Also, since the diffusion coefficient of oxygen through water is less than through air by a factor of about 10,000, air films would have to be enormously thicker than liquid films in order to become controlling. Even this last possibility is made unlikely by the relatively low viscosity of air compared to water (roughly 1:50).

Maxon and Johnson (49) have suggested that while a liquid film may be rate limiting in the absorption of oxygen by sterile broth, a gas-film resistance sets the rate for actively respiring yeast cultures. They noted a correspondence between the oxygen uptake by yeast and by sulfite solutions. However, Miyamoto and his co-workers (54) have shown rather conclusively that absorption into sulfite cannot be gas-film controlled. The Japanese investigators used pure oxygen so as to eliminate any gas film, and they also observed temperature coefficients which were much too high to fit a gas-film hypothesis. Perhaps for yeast as for sulfite solutions, a major resistance is at the interface.

It is of more than theoretical interest to examine the individual resistances. If a gas film does fix the pace, then our present fermentation equipment should be redesigned so as to create more turbulence in the gas phase and thereby to reduce film thickness. If, on the other hand, a major resistance is at the interface, stirring will be of little account except as it creates more interfacial area.

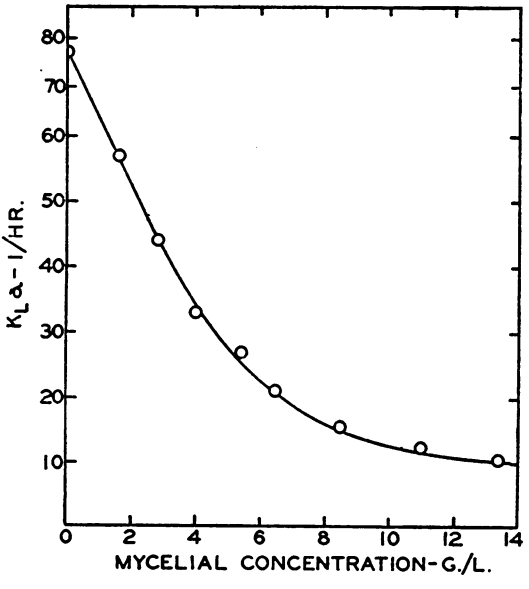

Figure 1. Effect of mycelial concentration of *Penicillium chrysogenum* on the absorption rate of a typical 5 liter stirred fermenter. (Replotted from a curve in Deindoerfer's unpublished thesis.)

The factors which affect K_La are listed by Hixson and Gaden (26) as the area of gas-liquid contact, the time of contact, and the agitation intensity. These in turn depend upon the design and operation of the equipment and upon such physical properties of the culture fluid as viscosity and surface tension. Hixson and Gaden observed that after six hours the absorption rate constant for a small yeast propagator almost doubles its initial value. This variation in K_La they ascribed to the noticeable physical changes in the medium which occurred during the course of growth. In some recent work from Gaden's laboratory these changes are further elucidated (unpublished results of F. Deindoerfer). For example, figure 1 shows how rapidly K_La decreases as the concentration of mold mycelium increases. The effect is undoubtedly caused by changes in the viscosity of the fermentation mash.

III. METHODS FOR INCREASING THE TOTAL RATE OF GAS ABSORPTION

The rate at which oxygen goes into solution can be increased either by improving K_La or by raising the partial pressure of oxygen in the gas so as to create a larger driving force, $C^* - C_L$. These methods will now be discussed. The operating and design variables which affect K_La are agitation, type of sparger, and gas velocity.

1. *Agitation.* A general review of the literature on agitation up to 1944 was given by Hixson (55) as part of an engineering symposium on the subject. More recent developments have been covered in a series of annual reviews which have appeared since 1946 in the January issues of *Industrial and Engineering Chemistry*. Of greater utility perhaps to the microbiologist who wishes a quick survey, are articles by Rushton (56) and by Mack and Uhl (57). Agitation acts to improve K_La in three ways: First, by chopping up the air stream into small bubbles, agitation increases the interfacial area, a. Second, by circulating the liquid in swift eddies, it delays the normal escape of bubbles. Lengthening the time of contact in this manner has the effect of increasing the interfacial area (58). Third, agitation creates turbulent

shear which reduces the thickness of the liquid film. Insofar as diffusion through the liquid controls, such shear increases K_L.

Because these three effects cannot be separated, we resort to empirical correlations between the degree of agitation and the absorption rate constant. Cooper, Fernstrom, and Miller (46) found that in a well designed system K_La is almost directly proportional to the power input per unit volume

$$K_La \propto (\text{power/volume})^{0.95} \quad (8)$$

This relationship, which is valid for both laboratory and plant-scale equipment, was confirmed by Karow, Bartholomew, and Sfat (50).

Power input per unit volume is a reliable index of the degree of agitation only if there is fully developed turbulence within the fermenter and no loss of power due to swirling or vortexing of the liquid. The criterion of turbulence is a dimensionless quantity, called the Reynolds number, which is defined as

$$N_{Re} = \frac{L^2N\rho}{\mu} \quad (9)$$

where L is impeller diameter, N is revolutions of impeller per unit time, ρ is fluid density, and μ is fluid viscosity. If the Reynolds number is above 10^5, the following general equation can be used to relate the horse-power to operating variables

$$\text{Power/volume} = cN^3L^5\rho \quad (10)$$

The constant c depends upon the particular impeller design. Equation 10 holds at all values of N_{Re} in the range of fully developed turbulence. Note that under these conditions the power input is independent of viscosity. Power input measurements are difficult to make, especially on large equipment, but the relative effects on K_La of changing the rpm and impeller diameter can be predicted from equations 8 and 10.

All fermentation vessels, even the simplest laboratory ones, should be adequately baffled. Unless this is done it is meaningless to cite a stirrer speed since the relative velocity between liquid and impeller changes with the extent of the swirl. At a given speed it has been found that adding baffles causes a rapid increase in power consumption up to a point, but the addition of still more baffles does not increase the power consumption very much. Several "fully baffled" arrangements are described by Mack and Kroll (59); a standard one consists of four baffles each $\frac{1}{10}$ to $\frac{1}{12}$ of the tank diameter, and extending the full depth of the tank.

The following broad statements concerning the design of stirred tanks are intended primarily as a guide for microbiologists who desire to construct their own small fermenters. Turbines or paddles having a radial flow pattern are generally preferred to propellers, which produce axial flow. The impeller is located from a half to one diameter above the bottom of the fermenter, and its diameter is about one-third of the tank diameter although for viscous mashes the impeller diameter is often increased beyond the one-third ratio. Air is admitted through a sparge ring having a diameter about three-fourths that of the impeller; at air rates less than 150 ft/hr, however, a single center inlet directly beneath the impeller gives equally good results. Multiple impellers are separated on the shaft by a distance of one impeller diameter, and the height of liquid is approximately one tank diameter in the "standard" arrangement. Figure 2 illustrates a typical stirred tank with "closed" turbines, *i.e.*, with turbines having a disc or baffle transverse to the plane of the blades.

In scale-up from laboratory to factory the usual procedure (46, 50) is to insure geometric similarity of the fermenters and equal power input per unit volume. Not all dynamic and geometric factors can be kept the same, however, (*e.g.*, tip velocity of the impeller is not held constant in the above method), and large extrapolations of the data are unwise. Enough power must be available to run the impellers in ungassed culture fluid, but during the sparging much less than rated power is drawn owing to the low density of the bubbly mixture (46, 48). At high gas rates, power input may be only one-fourth of the ungassed value.

When agitation is defined only in terms of power input per unit volume, no account is taken of how the power is distributed within the fermenter. The inadequacy can be illustrated by comparing a small high-speed stirrer with a large, slow-moving one operating at the same power. The former may effectively disperse the air stream into fine bubbles, but it fails to circulate these through the mash. A slow mixer, on the other hand, is unable to shear off the air

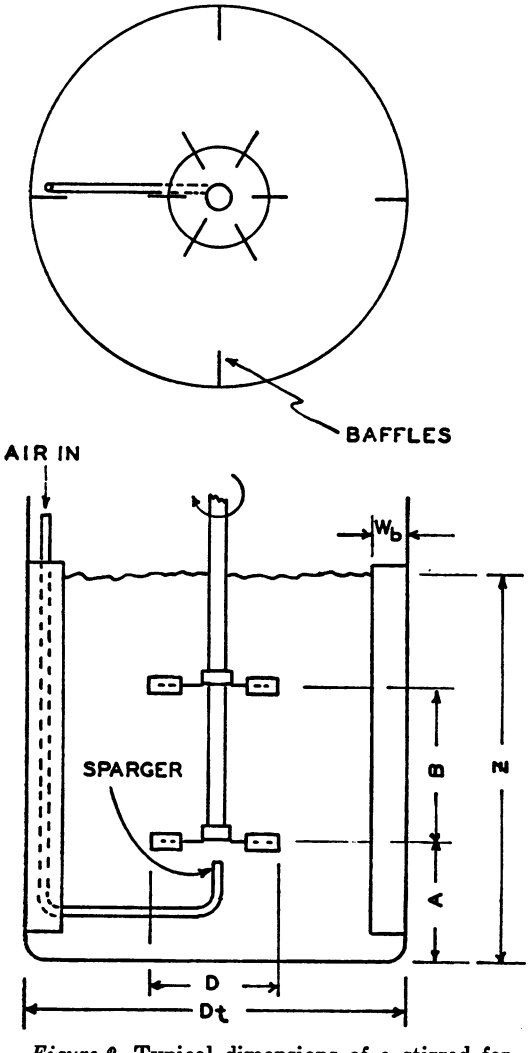

Figure 2. Typical dimensions of a stirred fermenter: $Z/D_t = 1.0$; $D/D_t = 0.34$; $A/D = 0.8$ to 1.0; $B/D = 1.0$ to 1.2; $W_b/D = 0.08$ to 0.10.

bubbles even though it provides good pumping action. Just what compromise to arrive at in the optimum design of tank and impellers would have to be worked out separately for each type of fermentation, and no rational approach is yet available. Added refinements in design will depend ultimately upon a better understanding of transfer resistances and the mechanics of bubbles.

2. *Gas velocity.* For stirred tanks it has been found that K_La varies as $V_s^{0.67}$ (46), where V_s is the superficial gas velocity (*e.g.*, cm/sec or ft/hr based on the total cross-sectional area of the vessel). Small laboratory fermenters may exhibit a slightly different characteristic in which the exponent of V_s is 0.4 to 0.5 (46, 49), but otherwise the relationship appears to be quite general. For an unstirred propagator Hixson and Gaden (26) found the exponent to vary from 0.33 to 0.82 depending on the fineness of air dispension, and elsewhere in the literature a value of 0.74 is reported for apparatus which is not stirred (60). Mechanical effects of the air bubbles cause these variations, more turbulence being created by larger bubbles as V_s is increased.

At high air rates, flooding or "loading" of the impeller occurs. Cooper, Fernstrom, and Miller (46) reported that above a superficial velocity of about 400 feet per hour their vaned-disc impeller seemed to be unable to disperse the air. Bypassing of the impeller is accompanied by escape of the gas in large bubbles around the shaft, and above the loading velocity K_La does not increase much with further increases in gas rate. Open-type turbines or paddles load at low air velocities because the gas can more readily rise up through the slow-moving central part of the turbine. For example, Cooper, Fernstrom, and Miller observed loading of a paddle stirrer at a velocity of only 70 feet per hour.

Lee (61) has listed several methods which have been used for reporting the degree of aeration, but it should be emphasized that of these, only the linear velocity, V_s, is appropriate. The commonly used "volumes of air per volume of medium per unit time" is wholly unrelated to gas holdup or K_La (44); with such a measure of aeration, experimental results cannot be reproduced by other investigators who employ "the ame gas rate".

3. *Type of sparger.* Comminution of air may be achieved by methods other than high-speed agitation, and as pointed out by Hixson and Gaden (26), unstirred vessels may offer economy of operation if the breakup of cell clumps is not demanded. A review of fine-bubble aeration by de Becze and Liebmann (62) describes the many ingenious devices which have been used for this purpose.

The effect of such factors as orifice diameter, gas velocity, and surface tension on bubble size and bubble velocity is under study both in this country (63, 64) and in England (65, 66). Most of the work concerns the mechanics of a single stream of gas bubbles and is not immediately useful for practical aeration problems. For

bubbles from a single orifice, bubble volume is directly proportional to orifice diameter and surface tension, and it is inversely proportional to the density of the liquid (67). Neither gas pressure, temperature, nor liquid viscosity is reported to have much effect on bubble size, but unfortunately no work has been done with systems exhibiting non-Newtonian viscosity (plastic flow) such as occurs in mold cultures (50).

Mass transfer from a stream of single bubbles was reported by Coppock and Meiklejohn (67) in terms of K_L values since the interfacial area was accurately known. The absorption coefficient was found to vary directly with bubble velocity, and it was immaterial whether oxygen or air was used in the gas phase. Values of K_L ranged from 100 to 200 cm/hr for absorption into deaerated water. These values were some five times smaller than those estimated from data by Pattle (68). There is general agreement that coalescence of bubbles occurs mostly by head-on rather than sideways collision, and that a bubble may be pushed into the slipstream of another bubble immediately ahead of it, thereby causing such contact. Small amounts of the same materials which promote foaminess inhibit coalescence.

Observations on swarms of bubbles (69) show that the gas holdup increases as expected with increasing gas rate, but that in unstirred vessels the initial linear relation is interrupted at some critical rate which corresponds roughly to the loading point for stirred vessels. Bubble size is always larger than the pore size by a factor of 10 to 100 for porous spargers. Surface-active agents which are present in most complex media reduce the bubble size, and one might suppose that the resulting increase in interfacial area would cause higher values for K_La. On the other hand, tiny bubbles which are unable to create much turbulence in their passage through the broth would be surrounded by rather thick liquid films, and these in turn would adversely affect K_L. It has also been suggested (27, 70) that the presence of large organic molecules at the gas-liquid interface interferes with the passage of oxygen gas, but others (51, 54) discount such an effect. One can state only that the influence of surface-active agents on mass transfer is not yet clear. Bartholomew *et al.* (27) found that surface-active materials tended to lower the absorption rate of water, while Hixson and Gaden (26) observed an opposite effect on K_La during the growth of yeast. The performance of porous ceramic and porous carbon spargers has been described in general terms by Unger *et al.* (71), and more quantitatively by Anderson (72) and King (73) who used sulfite absorption tests. Such spargers are used for aerating sewage and for yeast production despite their tendency to clog and become less efficient during long periods of service.

Air is dispersed in another method by its introduction at acoustical velocity as proposed by Achorn and Schwab (74). These investigators found that with an orifice 0.00312 inches in diameter in an air tube 0.156 inches in diameter, many bubbles were less than 10 microns in diameter provided the pressure drop across the orifice was 13 pounds per square inch. In their laboratory apparatus they observed more violent agitation than was obtainable with porous media.

Perforated pipes have been widely used either alone or in conjunction with mechanical stirring. The pipe spargers are usually in the form of rings or crosses, with drilled holes ranging in size from 0.03 to 0.13 inches. Pressure drop along a pipe manifold causes nonuniform emission of air, and ideally finer holes should be drilled close to the air source (75, 76). Plugging of some of the holes, which is bound to occur in time, causes uneven distribution of air or perhaps local flooding of an impeller if the tank is stirred. A number of fermentation plants now prefer open or slightly constricted pipes for introducing the gas, and they depend upon intense agitation to give small bubbles and favorable transfer rates (27).

If air is sparged at high velocity through fine orifices, it does mechanical work on the fermentation mash, and power input from this source should be added to that from the agitator itself when scale-up of equipment is being considered or when equation 8 is used. Bartholomew *et al.* (27) describe the calculation of work done by the air stream.

4. *Increasing the driving force.* One obvious means of increasing the rate of oxygen supply to a culture medium is to raise the solubility, C^*, by increasing the partial pressure of oxygen. This may be done by using either oxygen-enriched air or air under pressure. Except for (a) the sweeping effect of the nitrogen, (b) the concomitant increase in carbon dioxide solubility as P is raised, and (c) a possible failure to supply enough

carbon dioxide along with the pure oxygen, the two methods for increasing C* are equivalent. Microorganisms are not injured by pressures up to 5 atmospheres (77) which is about the limit of practical operation. Oxygen or air under pressure has been found especially beneficial in gluconic acid (78) and citric acid (29, 79) fermentations which are characterized by unusually high oxygen demand.

Harmful effects of high oxygen tension have been noted many times, and no simple explanation will fit all cases; Bean (80) has exhaustively reviewed the literature up to 1945. On the one hand, poisoning may be reversible; if so, oxygen and reduced substrate compete for the same site on the enzyme (81), or else sulfhydryl groups on the enzyme itself are reversibly oxidized. On the other hand, continued exposure results in permanent damage to the respiratory enzymes. The cytochromes appear to be rather resistant, (82) and although flavoprotein activity increases with oxygen tensions up to 760 mm (83), higher pressures do cause damage (84) perhaps owing to accumulation of peroxide. The ease of poisoning depends upon the particular organism, and indeed with its environmental and nutritional status (85). Poisoning by oxygen has been noted in the production of yeast (86), acetic acid (32), and penicillin (30).

IV. DEMAND-SIDE RESISTANCES

1. *Liquid film around cells or clumps.* For single cells at least, a liquid film at the cell wall offers no appreciable resistance to the diffusion of oxygen. Occasionally though, microbiologists raise the question of such a resistance and express their concern over it. For example, Marshall *et al.* (87) state, "The rate of aeration employed in these studies was sufficiently rapid to maintain high extracellular O_2 tension. Limited diffusion of O_2 into the cell, however, cannot be excluded." The fact that above a critical oxygen concentration the uptake by microorganisms is independent of C_L may be taken as evidence that a liquid film surrounding each cell offers negligible resistance. Further proof comes from a calculation such as the following.

Consider a yeast cell 5 microns in diameter suspended in an infinite quantity of stagnant fluid. It has been shown theoretically and experimentally (41) that under such conditions

$$\frac{k_L D_c}{D_v} = 2 \qquad (11)$$

where k_L is the absorption coefficient for a liquid film, D_c is the diameter of the yeast cell, and D_v is the diffusivity of oxygen. Since $D_v = 1.8 \times 10^{-5}$ cm:/sec, k_L is 0.072 cm/sec. The ratio D_v/k_L can be considered a "film thickness", which here is equal to the cell radius. If the true rate of oxygen uptake is about 0.08 g/(hr) (g DW), as found by Hixson and Gaden (26), and if 25 per cent dry weight is assumed, each yeast cell consumes 4.1×10^{-11} mM O_2/hr. The maximum concentration difference between the bulk of the fluid and the cell wall is, by analogy with equation 3,

$$(C_L - C_W) = \frac{\text{rate of consumption}}{k_L a} \qquad (12)$$

where C_W is the concentration of O_2 at the cell wall. For a 5 micron yeast cell a is 7.86×10^{-7} cm², and the maximum concentration difference is therefore

$$(C_L - C_W) = \frac{4.1 \times 10^{-11}}{1} \left| \frac{1}{0.072} \right| \frac{1}{7.86 \times 10^{-7}} \left| \frac{1}{3{,}600} \right| \frac{1{,}000}{1}$$

$$= \frac{\text{mM } O_2}{(\text{hr})(\text{cell})} \left| \frac{\text{sec}}{\text{cm}} \right| \frac{\text{cell}}{\text{cm}^2} \left| \frac{\text{hr}}{\text{sec}} \right| \frac{\text{ml}}{\text{liter}}$$

$$= 2.0 \times 10^{-4} \text{ mM } O_2/\text{liter}.$$

This difference in concentration is negligibly small compared to the level of C_L ordinarily maintained in the liquid (*e.g.*, C* for air is about 0.2 mM/liter). The liquid film is of even less importance for cells smaller than yeast because $C_L - C_W$ varies roughly as D_c^2.

Quite apart from the foregoing, one can predict that agitation in excess of what is required to suspend the single cells uniformly will not markedly improve the oxygen transfer from liquid to cell wall. The reason is that the thickness of the liquid film surrounding each cell can be reduced only by increasing the *relative* velocity between the cell and the fluid. Now the cells are so small and their specific gravity is so close to

that of the culture medium that they possess insufficient inertia to attain an appreciable velocity relative to the medium. In other words, the particles tend to follow the fluid streamlines, even though the latter may turn sharply and whirl about in swift eddies.[3] Despite all these considerations, studies on the liquid films around cells continue to be made, and these have been reviewed by Gaden (88). Most investigators are probably observing side-effects of agitation when they report improved growth rates and the like. For example, stirring will relieve supersaturation of CO_2 in the culture medium.

For cell clumps, such as occur in submerged mold fermentations, the liquid films may assume importance. Here, however, relative motion between the clump and its surrounding fluid is more likely. Furthermore, the intraclump resistance transcends that of the liquid film.

2. *Intracellular and intraclump resistances.* The most complete reviews on diffusion through deep layers of respiring tissue are those of Gerard (89), Jacobs (90), and Rashevsky (91). Calculations show that clumps of tissue must be less than about 1 mm in diameter if anaerobic conditions at the interior are to be avoided. The critical size depends, of course, on the Q_{O_2} of a particular tissue and on the presence or absence of an enclosing membrane of low permeability (92).

No quantitative information is available on the degree of agitation which is necessary to break down cell clumps although cell physiologists have made preliminary investigations on the effects of shear on tissues of higher plants and animals (93). Perhaps the most satisfactory progress, both in the theory and technique, is being made by those who are studying paper pulp suspensions. The work of Mason (94) and his associates, for example, is especially pertinent for those who are interested in the filamentous fungi.

V. PRACTICAL APPLICATIONS

The practical aspects of agitation-aeration are covered in reviews on fermentation published each year in September in *Industrial and Engineering Chemistry*. No complete survey will be attempted here, especially since the data are often too fragmentary to be useful.

1. *Performance of equipment.* For aerobic processes carried out in pilot-plant or full-scale industrial equipment, power input may vary anywhere from 0.1 to 1.0 horsepower per 100 gallons, and air velocities from 50 to 200 feet per hour, depending upon the tendency to clump and to foam as well as upon the oxygen needs of the culture. Some typical values of K_La for shake-flasks and other types of apparatus are collected in table 4. Smith and Johnson (94a) have recently presented a similar tabulation of effective aeration rates for the types of equipment used in their laboratory. They found that K_La values for shake-flasks could be increased fourfold by indenting the sides of the flasks.

It appears that if shake-flask contain only a shallow layer of culture medium, they are superior to simple bubbling devices. Stirred fermenters give the best aeration. One cannot rely, however, on the absolute values of K_La shown in table 4 because undesignated factors come into play. For example, if shake-flasks develop a head of foam much of their effectiveness is lost (97).

Only a small per cent of the oxygen supplied to a fermenter is actually absorbed. Oxygen efficiency seldom exceeds 2 per cent under ordinary operating conditions, and it is often below 1 per cent. Low efficiency is not so serious because sterile air, while not free, is a relatively cheap raw material. Special devices can sometimes accomplish efficiencies of 30 per cent or more (68), but such high values are attained at the expense of a large pressure drop and low throughput of air.

On the other hand, a high oxygen efficiency may dictate the choice of a tall fermentation vessel rather than a short one. To illustrate this difference, let us suppose that a new fermenter is to be built with double the capacity of a present one. If the present vessel is of standard dimensions, with liquid height equal to the tank diameter, two possibilities which arise are: (a) the new vessel may be scaled up by a linear factor of $\sqrt[3]{2}$ so as to have twice the volume, or (b) it may be made twice as tall as the present one without any change in the diameter. For the latter case, Cooper, Fernstrom, and Miller (46) found that K_La is increased by a factor of about

[3] It should be stated of course that each particle rotates at an angular velocity which does increase with the degree of turbulence and which is *independent* of the size and specific gravity of the particle.

TABLE 4

Typical values of K_La[a] for various types of apparatus

Type of Fermenter	Agitation	Air Rate	K_La hr^{-1}	Reference
		ft/hr		
A. Shake type				
1. Respirometer vessel, 25 ml containing 3 ml (1 mm liquid depth)	2.4 cm throw 150/min	—	8.3[b]	95
2. Erlenmeyer flask 300 ml/one liter flask	7 cm throw 9/min	—	24	44
3. Erlenmeyer flask 75 ml/250 ml flask	1½ in. eccentric 220 rpm	—	26	15
4. Erlenmeyer flask 50 ml/500 ml flask	1 3/16 in. eccentric 210 rpm	—	90[c]	29
5. Erlenmeyer flask 50 ml/500 ml flask	1⅛ in. eccentric 253 rpm	—	*ca* 200	45
B. Bubblers—unstirred				
1. Model of sewage aerator (porous plate in a tank)	None	60[d]	5–9[e]	73
2. Glass column, 5 cm diameter (1 in. coarse sintered disc)	None	150	30	44
3. 2 liter glass vessel, 6 in. diameter				
Single orifice, 0.060 in. diameter	None	60	13	26
		120	22	
Fritted stainless steel, 2½ in. diameter, 65 μ openings	None	60	23	26
		120	30	
4. Single orifice (uniform stream of single bubbles, 3 mm diameter each)	None	0.065	0.86	67
C. Bubblers—stirred				
1. 6 in. diameter vessel, standard design	Single impeller 3 in. diameter 500 rpm (0.5 HP/100 gal)	60	420	27
2. 6 in. diameter vessel, standard design[f]	Single 4 in. impeller			49
	500 rpm	60	325	
	750 rpm	60	1,000	
	1,680 rpm	60	2,650	
3. 6 in. diameter vessel, standard design	Single 4 in. impeller 740 rpm	65	420	15
4. 36 in. diameter vessel, standard design (120 gallon working capacity)	Single 10 in. impeller 300 rpm	50	600	96
5. 15,000 gallon vessel	0.2 HP/100 gallon	175	370	46, 50

[a] Values are only approximate since often they were obtained by interpolation from published diagrams. Most experimenters used sulfite method of evaluation.

[b] This value seems exceedingly low. The experimental technique may have been subject to large errors.

[c] With mycelium present.

[d] Based on area of sparger. A superficial velocity cannot be calculated. Air rates 3 to 4 times as high are used in activated sludge tanks.

[e] Depends on the permeability rating of the porous plate. The sulfite method used to find K_La was not well conducted.

[f] Liquid height only ½ of vessel diameter. If height of liquid had been equal to diameter of vessel, K_La would have been only about 60% of values shown.

1.4 at a given linear gas velocity. Therefore the two schemes compare as follows if superficial gas rate and power input are held constant during the scale-up:

	Factor of increase using *Scheme A*	*Scheme B*
K_La	1.0 ×	1.4 ×
Total power	2.0 ×	2.0 ×
Volumetric gas rate	1.6 ×	1.0 ×
Oxygen efficiency	1.26 ×	2.8 ×

Less total air is required if the tank is made taller (scheme B), because the air is being scrubbed more thoroughly in its passage through the mash. Any improvement in K_La from still further increases in height is not large, and there are in addition engineering limitations on the use of very tall tanks. It might be supposed that the increase in gas pressure due to hydrostatic head would favor the solution of oxygen, but such an effect is almost cancelled by the smaller bubble size (50). In tall tanks where more than one impeller is necessary, it has been suggested that better absorption is possible if each impeller is gassed individually (50). More studies are needed on the effect of impeller position and D/D_t ratio before such suggestions can be accepted as final.

2. *Criteria of sufficient aeration.* The only way to be sure that the aeration is sufficient in a particular setup is to measure the concentration of dissolved oxygen. If a microbial culture fails to respire more rapidly as C_L is increased, then it may be safely assumed that the critical oxygen concentration has been exceeded and all is well.

Indirect methods of assessing C_L fail to give reliable information. Two examples of this will be cited. In a pilot-plant study of penicillin fermentation, Stefaniak and his associates (30) observed that beyond an aeration rate of 1 volume per minute per volume of culture, there was little increase in the rate of respiration, and they concluded that, "At aeration rates in excess of 1 volume per minute, available air was no longer the chief factor limiting oxidation rate." Such a conclusion may be correct, but an alternative explanation is that air in excess of 1 volume per minute was largely wasted. The latter possibility is strengthened by noting that the impeller was probably loaded at the limiting air rate of 200 liters per minute. Although no diameter was given for the 100 gallon tank, the superficial gas velocity must have been about 100 feet per hour. This exceeds the loading velocity of an open type impeller (46), and it is therefore not surprising that further increases in air rate were without effect.

Another example concerns the use of Warburg apparatus. It is commonly assumed that if more rapid shaking fails to increase the oxygen uptake, then diffusion into the liquid is no longer rate limiting. Such an argument presupposes that K_La increases without limit as the shaking is intensified. Winzler (14), who used both a manometric and a polarographic technique, showed quite clearly that diffusion could be rate limiting even though the Warburg cup was shaken to excess. Williams and Wilson (97a) made similar observations in their experiments with *Azotobacter vinelandii*.

3. *Relations between respiration and product formation.* Throughout this review respiration rate has been looked upon as a primary measure of the effectiveness of aeration. We must now examine how respiratory activity correlates with other properties of microorganisms such as their ability to grow and to manufacture useful or interesting products.

Empirically there is good correlation. Increases in K_La give rise to parallel increases in productivity, at least up to a point beyond which some process other than gas absorption becomes controlling.[4] Data on the formation of yeast cells (45, 98), bacterial cells (33, 94a), penicillin and streptomycin (27, 44, 50), organic acids (29), and mold amylase (29) all follow such a course. However, Calam, Driver, and Bowers (31) point out that the relationship is not a rigid one. They found, for example, that yields of penicillin cannot be predicted from knowledge of the respiration rate alone because each particular strain of mold, each type of fermentation apparatus, and each medium have a characteristic influence on the correlation. To emphasize the independence of respiration, growth, and penicillin formation, they cite the different temperature coefficients which they observed for these rate processes in *Penicillium chrysogenum*. Thermal increments, μ, were: for growth of mycelium, 8,230 g-cal; for respiration, 17,800

[4] Alexander and Wilson (33) and Smith and Johnson (94a) have emphasized that when aeration is adequate, the normal levels of sugar and minerals may be insufficient for optimum growth.

g-cal; for penicillin formation, 26,800 g-cal. Peculiar temperature breaks were also observed.

Other investigators have made similar observations. In their studies on ustilagic acid formation, Sallans, Roxburgh, and Spencer (96) found that to achieve the same productivity in shake-flasks with K_La = 90 (29) and in stirred fermenters, the latter had to be operated at K_La = 600. The values given for K_La in table 4 must therefore be interpreted cautiously when applied to product formation rather than to oxygen uptake.

Even with a given organism, apparatus, and culture medium a lack of correspondence exists between respiration and the formation of products. The point at which yield or rate of production reaches a maximum may not be always the same as C_{crit} for oxygen uptake. In shake-flask experiments, Shu (29) found that with citric acid (*Aspergillus niger*) and ustilagic acid (*Ustilago zeae*) maximum production did indeed coincide with maximum respiration. But the rate of mold amylase formation reached its peak when the oxygen needs of the organism (*A. niger*) were still unsatisfied. Presumably the same would be true for enzymes like nitratase (99) and hydrogenase (100) which depend upon low oxygen tension for their formation and function. Still a third possibility is that aeration would have to be far in excess of that required for full respiration. Such a situation might arise if the formation of product demanded a high oxidation-reduction potential. One rather clear-cut example is available from the work of Rolinson and Lumb (101) who, working with spore inoculated cultures of *Penicillium chrysogenum*, found that lard oil was attacked in preference to lactose provided very high aeration rates were used.

In their studies on facultative anaerobes Dagley, Dawes, and Morrison (*e.g.*, 102) emphasize that oxygen acts in complex ways to control the utilization of substrates. Thus, aerobic rather than anaerobic deaminases are formed at high oxygen tension, and the type of deaminase in turn may alter the course of growth and product formation. Gale (103) has reviewed some of the earlier work on such directive effects of oxygen.

Many fermentation processes of industrial interest are characterized by a long delay between the growth and respiration phase and the product forming phase. During the latter, oxygen supply is seldom limiting because the rate of respiration is relatively low. Rolinson (15) observed that failure to meet the peak oxygen demand of *P. chrysogenum* during its growth phase caused permanent damage which was manifested later when penicillin was being synthesized by the mold. Upon maturity, oxygen starved cells had a lower level of respiration and lower productivity. There appears to be adaptive development of respiratory enzymes similar to that reported in facultative microorganisms. Moss (104) who presents data on the adaptive cytochrome system of *Escherichia coli* summarizes the work which has been done in this field which continues to be actively investigated (104a).

4. *Overagitation and overaeration.* From time to time there are reports that too much agitation (105, 106, 107) or too much aeration (45, 108) has interfered with the yield of a particular fermentation product. A direct relationship, however, between cause and effect is not always proved. To illustrate the difficulties of interpretation, let us consider an example where increased aeration causes evaporative cooling of the fermentation mash. It would seem that any deleterious effect should be charged to lack of temperature control rather than to excessive aeration. A direct measure of the temperature within the fermentation vessel is especially necessary when high-speed stirring is used because all the mechanical power supplied is ultimately dissipated as heat. Also, under vigorous aeration the fermentation may proceed so rapidly that pH changes or degradative reactions are accelerated. If tests of productivity are made only at a stated time, say 60 hours after inoculation, a false impression may be gained of the efficacy of aerating. Still another type of artifact associated with high air rates is the loss of volatile intermediates such as acetaldehyde (49) or the loss of CO_2 (109). An interesting example, in which the cause of adverse results was traced, is supplied by the work of Pfeifer and his associates. In an early study (105) it was believed that poor results in the riboflavin fermentation were caused by high air rates. Later work (110), which was done on a different fermentation process, demonstrated that excessive amounts of antifoam agent could account for the low yields.

There are, of course, harmful effects from over-

agitation or overaeration. The complex effects of oxygen have already been mentioned, but more should be said concerning the damage to cells from stirring. Outright rupture of the cells is appreciable only when there are abrasive particles present (111) or when there is cavitation (112). In the ordinary fermentation apparatus, cavitation is not severe even with rapid stirring. In the author's experience a 5 minute treatment in the Waring blendor failed to disrupt young mold cells. Aged cells are so much more fragile, however, that high-speed agitation may well influence their lysis. It would be desirable to know the limits of shear both for breakdown of cell clumps and for cell rupture; a start in this direction has been made by Ackerman (113) who provided quantitative comparisons of cell fragilities. The ability to withstand sonically induced cavitation appears to bear no relation to cell size. Thus *E. coli* and T-2 phage have about the same fragility, whereas baker's yeast is not always broken even in intense acoustical fields.

VI. SUMMARY

To provide quantitative descriptions of the efficiency of aeration, K_La values should be measured, preferably with the aid of a polarograph and under conditions which closely simulate an actual fermentation. If a polarograph is not available, K_La can be estimated by the method of Cooper, Fernstrom, and Miller (46) in which a 0.5 M sodium sulfite solution containing a copper or cobaltiquinolate catalyst replaces the fermentation medium. The rate of oxygen absorption is measured by titrating iodometrically the unoxidized sulfite ions after a 3 to 20 minute period of aeration. Results are expressed as millimoles of O_2 absorbed per liter per hour, which is K_LaC^* in the nomenclature used here. Replicate determinations should agree to within about 5 per cent. Because oxygen may not have the same solubility in a fermentation mash as in the sulfite solution under test (*e.g.*, fermentation may be carried out under pressure), it is desirable to report K_La values rather than K_LaC^*. If tests are made with air at 25 C, C^* for 0.5 M sodium sulfate is about 0.20, and therefore $K_La = 5K_LaC^*$.

To illustrate the application of K_La data, suppose that it is desired to grow *Escherichia coli* in nutrient broth at 37 C ($C^* = 0.17$) using shake-flasks with a measured K_La of 25 hr^{-1}. The rate at which oxygen will be supplied to the cells is

$$\text{Rate of supply} = K_La\,(C^* - C_L). \quad (13)$$

If 10 per cent of saturation is set as a lower limit for C_L in order to have a safe margin of dissolved oxygen, equation 13 becomes

$$\text{Rate of supply} = 25\,(0.17 - 0.017) = 3.8 \text{ mM } O_2/(\text{liter})(\text{hr})$$

Reference to table 2 shows that the calculated rate of supply may not satisfy the peak demand of an active *E. coli* culture, and therefore less culture fluid should be used in the shake-flasks or they should be shaken more violently so as to raise K_La.

The following is a check-list of suggestions for those who work with aerated cultures:

1. Measure K_La for all aeration devices.
2. Report the linear velocity of air flow, but also include dimensions of the fermentation vessel so that a volumetric air rate can be calculated.
3. Check to see that air rates do not exceed 400 feet per hour for closed impellers, 70 feet per hour for open or paddle impellers.
4. Provide adequate baffling in stirred tanks.
5. To be sure that aeration is adequate, make a direct determination of the dissolved oxygen within the medium.

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